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EFFECT OF *Bryonopsis laciniosa* FRUIT EXTRACT ON DIAGNOSTIC MARKERS IN DEN INDUCED HEPATOCELLULAR CARCINOMA IN RAT.

Biochemistry

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S. Mahalakshmi — Research Scholar P.G. and Research Department of Biochemistry, Rajah Serfoji Govt. College [Autonomous], Thanjavur-613 005. Thanjavur, Tamil Nadu, South India.

T. Malarvili — P.G. and Research Department of Biochemistry, Rajah Serfoji Govt. College

S. Velavan — Department of Biochemistry, Marudupandiyar College, Thanjavur, Tamil Nadu, South India.

INTRODUCTION

Cancer cells display a broad spectrum of alterations that include gene rearrangements, point mutations and gene amplifications, leading to disturbances in molecular pathways regulating cell growth, survival and metastasis. When such changes manifest in majority of patients with a specific type of tumor, these can be used as biomarkers for detection and developing targeted therapies, besides predicting responses to various treatments (Hanahan and Weinberg, 2000; Ludwig and John, 2005).

Every cell type has a unique molecular signature, referred to as biomarkers, which are identifiable characteristics such as levels or activities (the abilities of genes or proteins to perform their functions) of a myriad of genes, proteins or other molecular features. Biomarkers are therefore, an objective measure or evaluation of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention. This includes all diagnostic tests, imaging technologies and any other objective measures of a person's health status. Biomarkers are subject to dynamic modulation, and are expected to enhance our understanding of drug metabolism, drug action, efficacy and safety. These can also facilitate molecular definition of diseases, provide information (Anant Narayan Bhatt et al., 2010).

Technologies to recognize and understand the signatures of normal cells and how these become cancerous, promises to provide important insights into the aetiology of cancer that can be useful for early detection, diagnosis, and treatment. Biomarkers are therefore invaluable tools for cancer detection, diagnosis, patient prognosis and treatment selection6. These can also be used to localize the tumour and determine its stage, subtype and response to therapy. Identification of such signature in surrounding cells or at more distal and easily sampled sites of the body viz., cells in the mouth (instead of lung) or urine (instead of urinary tract) can also influence the management of cancer (Sawyers, 2008).

A major challenge in cancer diagnosis is to establish the exact relationship between cancer biomarkers and the clinical pathology, as well as, to be able to non invasively detect tumours at an early stage. Similarly, identification of subtle changes in the genomics and proteomic status specific to malignant transformation will allow molecular targets to be used for developing therapeutics. Biomarkers employed currently in clinical oncology for diagnosis and therapy as well as potential ones that particularly hold promise as targets for therapy (Srinivas et al., 2001). In the present study to evaluate the Bryonopsis laciniosa fruit extract (BLFE) on Hepatospecific enzymes such as transaminases (AST and ALT), ACP, 5' Nucleotidase, ALP, LDH, GGT and protein in DEN induced hepatocellular carcinoma rats.

MATERIALS AND METHODS

Animals

Male albino rats of Wistar strain approximately 4-6 months young rats (weighing approximately 180-220g) were used in this study. The animals were housed in spacious polypropylene cages bedded with rice husk. The animal room was well ventilated and maintained under standard experimental conditions (Temperature 27±2ºC and 12 hours light / dark cycle) throughout the experimental period. All the animals were fed with standard pellet diet (Gold Mohur, Mumbai, India) and water *ad libitum*. They were acclimatized to the environment for 1 week prior to experimental use. The experiment was carried out according to the guidelines of the Committee (BDU/IAEC/2015/NE/43/Dt.17.03.15) for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), New Delhi, India.

Collection of *Bryonopsis laciniosa* fruit

Bryonopsis laciniosa (Linn) fruits were collected from Ramanathapuram District, Tamil Nadu. The collected fruits were identified and authenticated by Botanist, Dr.V.Ramachandran. Associate professor, Department of Botany, Bharathiyar University, Coimbatore. A Voucher specimen (Number:BU/Dept BOT/Bl/16.06. 2014) has been deposited at the Herbarium, Bharathiyar University, Coimbatore, Tamil Nadu, India.

Preparation of extract

The collected fruits of *Bryonopsis laciniosa* were cut into small pieces and shade dried at room temperature and made a fine powder using pestle and mortar. The powdered materials of fruits were macerated with 70% methanol at room temperature for 24 hours. After 24 hours, the supernatant was transferred into china dish. The supernatant was completely removed by keeping the china dish over a boiling water bath at 45°C. A semi solid extract was obtained after complete elimination of alcohol. The obtained residue was kept in the refrigerator for further use. The extract was made upto a known volume in distilled water just before oral administration.

Experimental Design

The experimental animals were divided into four groups, each group comprising of six animals for a study period of 16 weeks. Group I, control rats (untreated) were fed with standard diet and water *ad libitum*. Group II rats were induced intraperitonially with DEN (100 mg/kg b.w.) once in a week for a period of 6 weeks. Group III rats were administered with *Bryonopsis laciniosa* fruit extract (BLFE) alone (500mg/kg b.w.daily) was administered by intragastrically for 10 weeks. To the group IV rats, Bryonopsis laciniosa fruit extract (500mg/kg b.w.daily) was administered by intragastrically to DEN induced group of rats after the proliferation of tumor from 6th week onwards up to 16 weeks. At the end of the experimental period, the rats were euthanized by cervical dislocation. The blood was collected witht EDTA as anticoagulant. Serum was separated for the estimation of various biochemical parameters.

Biochemical estimations

The serum GOT and GPT was estimated by the method of Reitman and Frankel (1957). The serum alkaline phosphatase activity was estimated by the method of Kind and King's (1954). Acid phosphatase activity was measured by Annon (1963) method. GGT was determine by the method of Ochei and Kolhatkar (2000). The activity of serum lactate dehydrogenase was measured by the method of King (1965). Protein was estimated by the method of Lowry *et al.,* (1951).

Results

The activity of AST, ALT, ALP and ACP in the serum of control and experimental animals is presented in Table 1. It is observed that administration of DEN (Group-II) to rats produced a significant increase in the activities of all the marker enzymes, the increase being two-fold for AST, ALT, ALP and ACP, when compared to control rats. Treatment with BLFE administration (Group-IV) significantly decreased the activities of these enzymes to normalcy.

The table 1 also depicts the activity of GGT, LDH, 5 'NT and protein in the serum of control and experimental animals . A similar increase in the activities of these enzymes was observed in serum of rats after administration of DEN (Group-II) when compared to control rats. BLFE treatment markedly decreased the activity of these enzymes in rats towards near normal when compared to DEN treated rats.

Table 1 Effect of *Bryonopsis laciniosa* fruit extract on diagnostic markers in control and experimental rats (serum)

S. no	Parame ter	Group I	Group II	Group III	Group IV
1	Protein	7.02±0.40a	4.2±0.28 b	7.36±0.48 a	6.56±0.35 a
2	AST	35.63±2.30a	97.53±6.50 b	47.92±2.80 a	52.28±4.65 a
3	ALT	26.78±1.50a	54.44±4.87 b	24.88±1.21 a	29.555±2.00 a
4	ALP	40.21±2.80a	96.52±6.30 b	42.0±2.54 a	46.21±2.98 a
5	LDH	78.65±4.90a	135.66±10.32b	76.56±4.02 a	83.33±5.60 a
6	GGT	5.78 ±0.35a	11.98 ±0.78 b	6.46 ±0.45 a	6.77 ± 0.48 a
7	ACP	27.02 ± 1.23a	48.56 ±2.65 b	26.17 ±1.65 a	33.85 ±2.31 a
8	5' NT	3.25 ±0.21a a	6.33 ±0.43 b	3.77 ±0.21 a	4.86 ± 0.28 a

Each value is expressed as mean ± SD for six rats in each group.

Mean values within the Row followed by different letters (Super-script) are significantly (p< 0.05) different from each other were comparison by Duncan's multiple range test (DMRT).

Protein g/dl, AST , ALT, 5'NT, ALP, LDH, GGT, ACP = IU/L.

Discussion

During carcinogenesis, some enzymes can be used as an biochemical indicator of tumor response to therapy (Thirunavukkarasu and Sakthisekaran , 2003). Hepatospecific enzymes were activated when hepatocellular damage gave rise to abnormalities of liver function and these enzymes are remarkably increased in HCC. One of the most sensitive and dramatic indicators of hepatocyte injury is the release of intracellular enzymes, such as transaminases, phosphatases and LDH in the circulation after DEN administration. The measurement of phosphatase activity is useful as an indicator of liver function (Padmakumaran *et al.,* 1998). In the liver, it is closely connected with lipid membrane in the canalicular zone, so that any interference with the bile flow, whether extra-hepatic or intra-hepatic leads to increased serum levels of ACP and ALP activities. Aminotransferases (aspartate transaminases (AST) and alanine transaminases (ALT)) are reliable marker enzymes of liver and they are the first enzymes to be used in diagnostic enzymology when liver damage has occurred (Whittby *et al.,* 1984). Among the macromolecules that leak from the damaged tissues, enzymes are released into the body fluids due to their tissue specificity and catalytic activity.

AST and ALT activities in serum are generally accepted as an index of liver damage and this tendency is also known to be distinct in rodents (Ha *et al.,* 2001). There was a good correlation between the activities of ALT and AST with tumor volume during therapy. Rocchi *et al.* (1997) reported that there was an increase in the levels of these transaminases activity in serum of HCC patients. In concurrent with the above findings an elevated serum aminotransferase activities were observed in animals bearing HCC. BLFE treatment significantly attenuated this alteration thereby showing its anticarcinogenic activity.

Elevation of alkaline phosphatase is one of the signs, suggesting space-occupying lesions in the liver. An increased activity of ACP and ALP was seen in blood serum of animals with HCC, this may be due to the disturbance in secretory activity or due to altered gene expression in these conditions. Development of tumor results in tissue damage that lead to the release of ALP into circulation (Iqbal *et al.,* 2004) and this enzyme level have been elevated in blood serum of the tumor-bearing animals and this elevation is significantly suppressed by the supplementation of BLFE in diet. GGT has been shown to play an important role in the metabolism of foreign substances and also during cell growth and differentiation (Thusu *et al.,* 1991) and is over expressed in tumor cells resistant to therapeutic drugs (Bailey *et al.,*2001). Experimental studies have shown that GGT was strikingly activated during the course of hepatocarcinogenesis induced by several hepatocarcinogens in animals (Fiala and Fiala, 1973); chemical carcinogens may initiate some systematic effects that induce GGT synthesis (Vanisree and Shyamaladevi, 1998). This elevation reflects the progress of carcinogenesis, since its activity correlates with tumor growth rate, differentiation and survival of the host (Koss and Greengurd, 1982); in concurrent with above findings there was an increase in the levels of GGT in the serum of animals bearing HCC. This elevation indicates the basic tumor burden and BLFE treatment significantly decreased the elevation .

5′ nucleotidase was found to be elevated in the animals with solid tumors (Ip and Daw, 1978). The increased activity of this enzyme seems to have originated from the proliferating tumor cells (Dao *et al.,* 1980). Elevated activities of 5 'nucleotidase in carcinoma of liver and leukemia were reported (Thirunavukkarasu and Sakthisekaran , 2003; Rosi *et al.,*1998). In our study correlatively increased activities of 5′nucleotidase were observed in blood serum of the carcinogen administered animals and this elevation is significantly inhibited in the animals treated with BLFE.

LDH is a fairly sensitive marker of solid neoplasm (Lipport *et al.,* 1981) and very high LDH levels correlate with treatment failure (Pui *et al.,* 1985); numerous reports revealed increased LDH activity in various types of tumors (Thangaraju *et al.,* 1998; Engan T, Hannisdal, 1990). The elevated levels of LDH may be due to its overproduction by tumor cells. Proliferating malignant cells exhibit very high rates of glycolysis, which subsequently lead to elevated LDH activity. The results of the present study are in agreement with literature data and show elevated levels of LDH in blood serum of the DEN administered rats and this elevation was attenuated in BLFE treated rats.

Earlier work in our laboratory confirms the fruit of *Bryonopsis laciniosa* appear to be rich in secondary metabolites,widely used in traditional medicine to combact and cure the various ailments. Flavonoids are found to be abundant in the fruit (Ramya *et al.,* June 2015) and they protect against allergies,platelet aggregation, microbial infections,ulcers,hepatotoxins and tumors(Okwe DE, Omodamiro OD.,2005). They are powerful antioxidants and free radical scavengers, which prevent oxidative cell damage and one of the important naturally available small molecules in our daily diet and have been considered for use as potential drug candidates for anticancer therapy (Velavan, 2015). The common dietary flavonoids Luteolin, Quercetin, Rutin and Genistein have received significant attention for their protective as well as potentially destructive anti-tumour, anti-cancer and anti-oxidant activities. Flavonoids can exert their effect through their interaction with phase I metabolizing enzymes (eg, cytochrome P450), which metabolically activate a large number of procarcinogens to reactivate intermediates Flavonoids can exert their effects through their interaction with phase I metabolizing that can interact with cellular nucleophiles and ultimately trigger carcinogenesis. Flavonoids inhibit the activities of certain P450 isozymes, such as CYP1A1 and CYP1A2 (Marchand et al., 2000; Tsyrlov et al., 1994) thus they are likely to have a protective role against the induction of cellular damage by the activation of carcinogens. Another mechanism of action is the induction of phase II metabolizing enzymes (eg, GST, quinone reductase, and UDP-GT) (Bu-Abbas *et al.,* 1998; Sun *et al.,* 1998) by which carcinogens are detoxified and eliminated from the body. This helps in explaining the

chemopreventive effects of flavonoids against carcinogenesis.

Results demonstrated that BLFE treatment significantly attenuated the increased activities of these enzymes. The fruit extract helps in parenchymal cell regeneration in liver, thus protecting membrane integrity and thereby minimizing enzyme leakage. This result suggested that BLFE possess potential hepatoregenerating activity and offers a platform for using this fruit as herbal alternative for liver cancer. The chemoprotective effect of BLFE may be associated with enhancing antioxidants activity and inducing apoptosis.G iven that there is no effective treatment measure for hepatocarcinoma, our findings may suggest a potential use of BLFE for hepatocellular carcinoma chemoprevention.

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